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By Asbjørn Gildberg

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Abstract:

This report presents a brief overview of the literature concerning the use of enzymes to modify or process marine raw material. Research and development in this field is described, while the main emphasis is placed on describing industrial utilization, both nationally and internationally. The conclusion is that enzyme technology is little used in processing marine raw material, while interest in such use is increasing, and that Norway is in the forefront in the fields of ensilage technology, selective tissue degradation and the use of enzymes in rapid-ripening of herring.

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FOREWORD

Enzyme technology can often be an alternative to conventional technology. When processing fish and other marine raw material, such technology could contribute to gentler treatment and increased product yield. Enzyme technology could also be used to produce products which cannot be produced by conventional methods. Norges Fiskeriforskningsråd (Norwegian Fisheries Research Council) tasked Fiskeriforskning (Fisheries Research) with preparing an overview of the development and commercial usage of such methods. This report will form the basis for discussion about which sectors Norway should focus on in this field.

Tromsø, 1991-12-12

Asbjørn Gildberg

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1. INTRODUCTION

The use of enzymes has long traditions, particularly in the processing and production of foodstuffs. Enzymes have been actively used to only a limited extent in the treatment of marine raw material such as fish, shellfish and algae. The effects of enzymes present in the raw material have, however, been used for a long time in ripening fish and production of products, such as fish sauce and fish silage, by hydrolysis.

In the ripening of fish, it is primarily the proteolytic enzymes from the digestive tract which are active. During storage of ungutted fish, for example in salt brine, the enzymes leach out into the belly and are diffused into the fish meat. The enzymes cause a moderate hydrolysis which changes the flavour and texture of the fish meat.

Fish sauce is a product with long traditions in South-East Asia (Amano, 1962). The raw material from fish or shellfish, preserved with salt, is stored at tropical temperatures until most of the protein has been hydrolyzed by proteases which exist naturally in the raw material. Here, too, enzymes from the digestive tract play the most important role (Orejana and Liston,

1981). After about six months' storage, the protein hydrolysate, which is rich in salt, is drawn off and used as a flavouring agent and nutritive additive, particularly in rice dishes. About 250,000 metric tonnes of fish sauce are produced annually (Stefansson and Steingrimsdottir, 1990).

Fish silage is a relatively new product where the digestive enzymes in the raw material also cause protein hydrolysis (Tatterson and Windsor, 1974; Raa and Gildberg, 1982). Fish silage is an acid-preserved fish raw material which hydrolyzes rapidly if the fish stomach is part of the raw material. The stomach sac contains pepsines which quickly break down proteins in an acidic environment. Ensilaging as a method of preserving and processing fish raw material was developed in Scandinavia in the 1940's-50's (Edin, 1940; Petersen, 1953; Gilberg, 1953). Today, there is a relatively modest production of fish silage in Scandinavia and a few other places, but interest is growing, particularly in connection with the use of by-products from fish farming. Fish silage is mainly used as animal feed.

2.

The addition of enzymes in treating marine raw material probably was first used in the tanning of fish skins (Kjell Midling, in conversation), but the production of fish protein

hydrolysates by the addition of enzymes has been going on much longer. This technology was developed in, among other places, Canada and India (Tarr, 1948; Freeman and Hoogland, 1956, Sripathy et al., 1963), but has probably had the greatest commercial impact in France for companies such as Production C.T.P.P. and Isnard Lyraz. Among other things, these concerns manufacture flavouring agents and fish feed (In, 1990, Anon. 1979).

Enzyme treatment of stickwater is another example of active enzymatic hydrolysis of fish protein. This method was developed in Denmark in the 1980's to reduce energy consumption during fish meal production (Jacobsen and Lykke-Rasmussen, 1984; Jacobsen, 1985).

With regard to selective degradation of tissue fractions, most of this work has been done in Norway and Iceland (Stefansson and Steingrimsdottir, 1990). The best known of these processes is probably enzymatic roe cleaning and enzymatic treatment of squid (Raa, 1986; Nilsen et al., 1989).

The active use of enzymes in the processing of marine raw material has, so far, been limited in scope. New literature, however, documents a growing interest in developing gentle enzyme

processes as alternatives to purely mechanical processes (Stefansson and Steingrimsdottir, 1990; Martin and Patel, 1991; Raa, 1991).

The following is an overview of available literature and data within the areas mentioned above. In addition, a number of other methods of using enzymes in the treatment of marine raw material will be described. However, it is important to emphasize that access to data regarding commercial processes is limited. It can therefore not be excluded that there may exist commercial applications of enzymes on marine raw material without that fact being public knowledge.

2. FISH SAUCE AND FISH SILAGE

The production of fish sauce and fish silage employs enzymes which are naturally present in the raw material so that the protein hydrolysates can be extracted. During ensilaging, oil can also be extracted as a by-product. In the production of fish sauce, experiments have also been carried out with the addition of enzymes to hasten the hydrolysis process.

The production of fish sauce is quite simple. Ground fish raw material or whole small fish having 20-40% sea salt added is stored at about 30°C in closed tanks 6-12 months after which a clear light brown protein hydrolysate can be drawn from the tank. Usually about half of the protein nitrogen can be extracted in this hydrolysate, while other remaining material is extracted several times with salt brine until most of the protein material and flavouring substances have been extracted. The extracts are sold as less expensive fish sauce products, graded according to the protein nitrogen content. According to Philippine standards, the highest quality fish sauce must contain at least 10% protein material, while the lowest quality must contain at least 3 % (Espejo and Magno-Orejana, 1980). Between these there are two other quality gradings.

It is mainly enzymes of the trypsin type which are active in the hydrolysis process (Orejana and Liston, 1981), but exopeptidases are also of great importance to the formation of flavouring components. Salt-tolerant and halophile bacteria are somewhat active even at very high salt concentrations (Larsen, 1986) and they are of great importance to the formation of aromatic components such as volatile fatty acids (Beddows et al., 1976,; Beddows et al.; 1980).

The high salt concentration in traditional fish sauce inhibits the autolytic process. Normally, sun dried salt is used, and it has been found that the rate of autolysis increases with the purity of the salt (Hamm and Clague, 1950), probably because impure heavy metals inhibit the activity of the proteinases.

It is often desirable to shorten the production time of fish sauce so that the need for storage capacity is reduced. This can be done in many ways. The simplest method is to increase the

4.

temperature during the first part of the storage period. Trypsin-type enzymes from tropical fish can tolerate relatively high temperatures (Gildberg, 1982), and a 1-2 week initial storage period at around 40° C can produce a good yield of sauce after a couple of months' storage (Hamm and Clague, 1950).

Trypsin-type enzymes are most active under alkaline conditions. If a small amount of salt (about 7%) is added to the raw material and made alkaline with soda-lye, the enzymatic action will be very effective and only a short initial storage under such conditions will produce a high autolytic yield (Gildberg, 1989). If the raw material is then neutralized with hydrochloric acid and more salt is added, the yield will be almost optimal after only a couple of months' storage.

Experiments with initial acidification have also been carried out (Beddows and Ardeshir 1979b; Gildberg, et al., 1984). This method is probably less suited since the acidification impairs most of the trypsin activity that should contribute to proper development of flavour after neutralization.

The rate of hydrolysis may be increased by the addition of plant enzymes. In Vietnam, this principle has also been used traditionally during the production of fish sauce from gutted fish. Here, pineapple juice rich in enzymes has been mixed with the fish to hasten the protein hydrolysis (Owens and Mondoza, 1985). In Thailand, some fish paste products are produced in a similar manner by mixing chopped pineapple or papaya with the fish meat (Phithakpol, 1989). These fruits contain bromelin and

papain, respectively, - proteinases which effectively hydrolyze fish protein under low acidic conditions. Lately, controlled experiments have been carried out by adding enzymes to increase the rate of hydrolysis during the production of fish sauce. Other experiments have been carried out using, among other things, papain, ficin and bromelain (Beddows et al, 1976; Beddows and Ardeshir, 1979a). A good hydrolytic yield was achieved after only 2-3 weeks' storage, but the development of flavour was not satisfactory. Nevertheless, when the hydrolysates were mixed with sauce produced in the traditional way, the flavour and aroma of the mixture was satisfactory. The best results were obtained with bromelain, a cysteinic proteinase extracted from pineapple stems. About 1% enzyme preparation and 0.04% cysteine was added to the raw material which was preserved with 40% salt (Beddows and Ardeshir, 1979a). Cysteine is added to stabilize the enzyme's active principle. Also in Indonesia, good results have been obtained when using pineapple enzymes in the production of fish sauce (Kumalaningsih, 1990). There are many indications that enzymes are now added during industrial production of some fish sauce products in Thailand (Chaufah Thongthai, in conversation).

5.

It is evident that the amount of enzymes is important to the rate of hydrolysis. If only fish viscera are used as raw

material, a rapid initial autolysis may be achieved. The proteinase activity decreases quickly, however, after only one week's storage. If the hydrolysate is drawn before the rapid decline has started, then it is possible to extract a concentrate of trypsin-type enzymes using ultrafiltration. This concentrate can be stored for a couple of months in a refrigerated room without the activity being drastically reduced. The permeate can be combined with the sauce fraction which occurs during further storage (Gildberg, 1992).

Ensilaging of fish raw material is an alternative to fish meal production. Ensilaging is less capital- and energy-intensive because, among other things, the raw material's own enzymes are utilized as tools to separate protein material from oil. These advantages were already described in a Norwegian patent early in the 50's (Gilberg, 1953) There is, however, an important difference between the proteins in fish meal and those in fish silage since the silage proteins are much more broken down into small peptides and free amino acids. This results in areas of application differing somewhat even though the raw material is often similar to start with (Lisac, 1961; Whittemore and Taylor, 1976; Strøm and Eggum, 1981; Raa and Gildberg, 1982; Hardy et al., 1984; Krogdahl, 1985a,b; Stone and Hardy, 1986).

If the enzymes in the silage are deactivated during an early phase of the autolytic process, the degradation of the protein will be considerably reduced, and the protein composition will be more like that found in fish meal. Such treatment may be desirable in some cases, but that is not the topic of this report.

6.

A number of acids and acidic mixes have been used in the preservation of fish silage, but most commercial production now uses 2-3% formic acid. If the oil is to be utilized, it is important that an antioxidant also be added.

In formic acid preservation, the pH is lowered to about 4. At this pH, the activity of the fish pepsins is high and causes rapid hydrolysis if the temperature is 20 - 40°C (Tatterson and Windsor, 1974); Wignall and Tatterson, 1976; Backhoff, 1976; Raa and Gildberg, 1976). It is, however, a prerequisite that the stomach sac of the fish form part of the raw material if rapid autolysis is to be obtained. If the pepsin content is low due to the feeding situation or the season of the year, then it may be appropriate to add enzymes or mix in enzyme-rich raw material. The addition of, for example, cod viscera may be a good alternative (Gilberg, 1953).

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Silage from pure fish viscera of carnivorous fishes autolyzes very rapidly, and the addition of protease preparations to such silage does not increase the rate of hydrolysis (Freeman and Hoogland, 1956). Most of the tissue material is broken down after a few days' storage so that about 60% of the volume can be drawn off as a quite clear yellowish-brown liquid phase with about 12% protein material (Gildberg and Raa, 1977). The remaining material is mainly present in the form of a protein fat emulsion on top of the liquid phase. More protein hydrolysate and pure oil can be extracted from this fraction after heating to 90°C and separation using a normal oil separator (Raa et al, 1983). Similar methods can also be utilized in the oil separation of ensilaged herring waste (Wignall and Tatterson, 1976). The liquid phase can easily be evaporated to 60% solids and will then be stable for storage and well suited as a protein component in feeds.

In ultrafiltration of the liquid phase from fish viscera silage, a pepsin concentrate can be extracted which can be dried and used for biotechnological purposes (Gildberg and Almås, 1986; Almås, 1990). If viscera silage is preserved by inorganic acids instead of formic acid, the permeate from the ultrafiltration will also be suitable as a nitrogen source for a number of micro-

organisms (Clausen et al, 1985; Almås and Sandsdalen, 1989; Vecht-Lifshitz et al., 1990, Almås, 1990).

7.

The ensilaging technology has been tested on other marine raw materials, including seal carcasses and krill. Ground-up seal carcasses preserved with formic acid autolyze slowly, but the enzymes nevertheless make the product pumpable and easy to mix with other feed components. Such feed is well-suited for fur animals (Stormo, 1983). Krill preserved by formic acid autolyzes and acquires a consistency that makes it well-suited as a component in fish feed (Geir Inge Auklend, in conversation).

3. PROTEIN HYDROLYSATES

This chapter will deal mainly with the active use of added enzymes to produce hydrolyzed protein from fish and shellfish.

Already in the 40's, Canadian researchers were experimenting with the development of such methods. Peptone (hydrolyzed protein) was manufactured by mixing proteolytic enzymes from fish with fish meat (Tarr, 1948). Later, peptone was manufactured from viscera of cod and haddock (Freeman and Hoogland, 1956). Pepsin, papain and other commercial proteases were used, but it was concluded that the addition of enzymes did not increase the yield since such raw material naturally contains large quantities of digestive enzymes. After 2-4 days' hydrolysis at 25-50°C, more than 80% of the raw material had broken down into water-soluble components regardless of whether enzymes were added or not. The hydrolysates could be dried by spray-drying, vacuum-drying or spin-drying. Spray-drying produced the lightest and finest product.

In India, experiments on hydrolysis of fish muscle with the aid of papain have been carried out (Sripathy et al, 1963). Ground fish muscle was mixed with equal parts of water and

commercial papain was added in an amount corresponding to 0.1% of the weight of fish. Hydrolysis was carried out at pH 5 and at 55°C, and the enzyme activity was stopped by boiling after 2 and 17 hours, respectively. After filtration, both hydrolysates contained the same amount of nitrogen, but the amount of amino nitrogen was considerably higher in the sample which had hydrolyzed the longest. Feeding experiments with rats found that this hydrolysate produced the highest degree of protein utilization. This is assumed to be because the tryptophan content was considerably higher in this hydrolysate. It has been found that aromatic amino acids and cystein are often freed relatively slowly during hydrolysis (Higashi et al, 1965; Raa and Gildberg, 1976). Tryptophan could act as a limiting amino acid in brief hydrolysis of fish protein.

Even in Japan, they have been studying fish protein hydrolysates for a long time. Yanase and Murayama (1965) found that enzyme hydrolysates of by-products from mackerel contained an unknown growth factor which produced a significant increase in growth among rats if 10% of the protein in a casein-based diet

9.

was replaced with fish protein hydrolysate. Other experiments found that the choice of protease may be very important to the nutritive value of hydrolysates. Boiled mackerel raw material

hydrolyzed with proteases from Streptomyces species was found to have higher nutritive value for rats than did casein, while hydrolysis with proteases from Bacillus subtilis produced a much lower nutritive value, probably due to an imbalance in the composition of the amino acids. (Higashi et al., 1965).

Plant proteinases and microbial proteinases are mainly used for hydrolysis of fish raw material (Mackie, 1974, 1982). Hale (1969) found that there was relatively little difference in the yield from hydrolysis when different proteinase preparations were used to hydrolyse heat-denatured protein from haddock fillets. Approximately 60% soluble material was obtained from a 24-hour hydrolysis using both the plant enzymes papain, bromelain and ficin with two different microbial proteinases and using mammalian pepsin and trypsin. A corresponding yield was obtained after 4 hours of hydrolysis of fresh cod muscle with ficin (Mohr, 1977; 1980). With large amounts of enzyme added (1% of the dry matter weight of the fish) the yield from hydrolysis could be increased to about 70%. However, this yield was obtained in a relatively thin muscle suspension. When the amount of water added equalled the amount of fish muscle, a condition

common in industrial processes, the yield was reduced to less than 60%. This is probably due to the effect of the enzymes being inhibited by the hydrolytic products.

Since fish muscle is, of course, a very complex protein raw material, and has a high content of protease inhibitors as well (Hjelmeland, 1983), it is quite difficult to explain the hydrolytic process in detail. Langmyhr (1981) studied the hydrolysis of cod muscle proteins with trypsin. Among the things he found was that the yield from hydrolysis was greatest if the enzyme was added at a low temperature and the temperature was slowly increased to the optimum for the enzyme's hydrolytic activity. This is probably because the protein had time to break down somewhat before it aggregated, and the size of the aggregates was therefore reduced so that the trypsin could more easily start working.

In industrial processes this effect would automatically be obtained if the enzyme were added before heating the raw material. It will always take a certain amount of time to heat up large volumes, and the enzymes will therefore have time to act before the protein coagulates.

10.

The functional properties of the hydrolytic preparations are

of great importance, particularly when they are to be used in foodstuffs. The solubility of the fish protein increases with the degree of hydrolysis, and the degree of hydrolysis increases with the duration of the hydrolysis and the amount of enzymes added (Mackie, 1982; Quaglia and Orban, 1987). Experiments in which sardines were hydrolyzed with the bacterial enzyme Alcalase showed that the ability to emulsify and surface hydrofobicity decreased as the degree of hydrolysis increased (Quaglia and Orban, 1990). Fish protein derivatives with good emulsive ability can be produced by acetylation, limited hydrolysis with bromelain, and defatting (Spinelli et al., 1975). Such preparations have been tested in many foodstuffs with good results. The best results were obtained when pure myofibrillar fractions were the basic material for derivation and hydrolysis.

Experiments in Malaysia have found that fish protein hydrolysate is well suited as a protein additive in biscuits (Yu and Tan, 1990). Tilapia muscle hydrolyzed with Alcalase was added and the best results, both functionally and by flavour, were obtained if the hydrolysate constituted 10% of the solids.

Mackie (1974) found that bromelain produces a more rapid hydrolysis of both fish muscle and fish waste than does trypsin.

Some results indicate that bromelain produces a better quality flavour in the hydrolysates than do most of the other proteinase preparations (Rutman, 1971; Beddows et al., 1976; Beddows and Ardeshir, 1979).

If fish protein hydrolysates are to be used in consumer products, then flavour and aroma are of great importance. It is well known that fish protein hydrolysates are usually very bitter. That is the main reason such products are not being used in consumer products in the U.S.A. (Peppler and Reed, 1987). There are, however, several U.S. patents which deal with the production of fish protein hydrolysates. Two of them describe methods which are supposed to produce products with no smell or bitter taste (Rutman, 1971; Ikeda et al., 1977). In one of the methods, where relatively lean fish is used, the fish is gutted, the head removed and skinned before the bones are removed and it is washed with water (Rutman, 1971). Some of the water is then removed by centrifuging and an emulsion of stabilized fat is mixed in during homogenization. Thereafter, bromelain at room temperature is added and the mass heated to about 55°C in 15 minutes. The temperature is then quickly increased to 80°C to inactivate the enzyme. Finally, the mass is homogenized and spray-dried. The finished product, which contains 70% protein and

11.

25% fat, is easily water soluble and produces a stable emulsion which, when stored for many days, did not separate.

The other patent (Ikeda et al, 1977) describes a two-step hydrolytic process where the raw material may be quite varied. Sardines, mackerel, pollock and krill are mentioned as the actual raw material. Whole fish with an equal amount of water added is heated to above 60°C to inactivate endogenous enzymes. After 15 min. the temperature is lowered to about 60°C and the pH adjusted to 9 before a commercial proteinase, which is active under basic conditions, is added. After one hour the pH is lowered to about 5.5 and a commercial proteinase, which is active under acidic conditions, is added. After an hour's hydrolysis, the material is separated in a three-phase separator so that an oil phase, a protein hydrolysate and a sludge phase, are extracted. The hydrolysate is filtrated and vacuum evaporated to 60-70% solids. Concentrates produced by this method will have no fishy smell or bitter flavour.

Typical for both of these methods is that relatively short hydrolysis periods are used. This reduces the formation of bitter

peptides. In the latter method, the raw material's own enzymes are also inactivated in order to reduce the formation of bitter peptides.

The problem of the bitter flavour can be reduced if the bitter peptides are further broken down by the use of special enzymes (In, 1990; Sugiyama et al.,1991). As a general rule, it can still be said that a brief, controlled hydrolysis should be used where flavour and functional properties are important. The selection of enzymes will also be of great significance, and bromelain appears to be the enzyme which is often used to produce hydrolysates intended for consumer products. Inactivation of endogenous enzymes by heating of the raw material may be necessary to avoid the wrong aroma or poor functional properties (In, 1990; Ikeda et al, 1977).

12.

In consumer products, rancidity of fat can also be a big problem. If a protein hydrolysate contains more than 1% fish fat, then the fat must either be removed or stabilized with an anti-oxidant to avoid a rancid flavour or the formation of harmful oxidation products (Mackie, 1982).

Though many variations of the method of producing fish protein hydrolysates have been described, industrial processes will, by and large, include the following steps:

1) The raw material is either chopped or ground up.

2) An amount of water equal to the amount of raw material is added before any pH adjustment.

3) A commercial enzyme preparation, usually extracted from plants or micro-organisms, is added before the temperature is raised to 50-60°C while stirring.

4) At the end of the hydrolysis, the enzymes are inactivated and the product is pasteurized by increasing the temperature to 80-90°C.

5) Oil, bones and sludge are separated from the protein hydrolysate using a three-phase separator.

6) The protein hydrolysate is filtrated, evaporated and dried by vacuum or spray drying.

It is difficult to get an overview of the extent of industrial production of fish protein hydrolysates. However, it

is known that much of the fish aroma preparations, fish soup powders and fish pastes available on the market are produced by enzymatic hydrolysis (Shoji, 1990; In, 1990). It is also evident that fish protein hydrolysates are included as ingredient in feed products for fish, domesticated animals and pets. The French companies Isnard Lyraz and Production C.T.P.P. are probably the leaders in this market. In France, efforts to develop such processes have been going on since 1963 (Noel, 1974). In addition, much interesting basic research has been carried out which has found that factors in fish protein hydrolysates may have immuno-stimulating effects (Vinot et al., 1989). These results, by the way, agree with observations made in Norway, where it is claimed that disease frequency was clearly reduced among domestic animals given fodder with fish viscera hydrolysate added (Sigmund Høydal, in conversation). In Norway, fish protein hydrolysates are manufactured by, among others, Rieber & Son, PRIMEX and Oddvin Bjørge A/S.

13.

With regard to growth substrates for micro-organisms based on hydrolyzed fish protein, Norwegian firms such as Marine Biochemicals A/S and PRIMEX have been world pioneers (Almås and Sandsdalen, 1989, Almås, 1990); Vecht-Lifshitz et al, 1990; Aksnes, 1990). Interest in the production of such products is,

however, on the increase in other countries also (Jassim et al, 1988; Levin et al., 1990). Most of the studies show that as sources of nitrogen for micro-organisms, fish protein hydrolysates are at least as good as peptone products manufactured from other protein sources. (Clausen et al., 1985; Gildberg et al., 1989; Vecht-Lifshitz et al., 1990).

Antarctic krill constitutes an enormous protein resource which has been little exploited. Since this krill has a high content of many different proteases (Osnes, 1985), it autolyzes very rapidly. Ellingsen and Mohr (1979) found that most of the protein in krill was broken down to free amino acids after two days at 20° C and, on the basis of their results, they have described an industrial process for the manufacture of free amino acids based on autolysis of krill.

Fish protein hydrolysate can, in certain cases, be an alternative to fish meal. In the U.S.A., fish protein hydrolysates are, first and foremost, used as weaning feed for piglets, as fish feed and as pet food. Pigs are given a spray-dried product costing NOK.15.- per kilo, while the fish feed producers use hydrolysate directly, or concentrates of hydrolysate, as an ingredient in the feed (Goldhor et al., 1990). About 50 billion kroner worth of cat and dog food is

sold in the U.S.A. annually. Fish protein hydrolysate is particularly used as a coating on cat food pellets to stimulate the appetite (Willard and Dayton, 1990). This coating constitutes about 3% of the weight of the food and would correspond to almost 20,000 metric tonnes of dry hydrolysate per annum if it were applied to all dried cat food sold in the U.S.A..

14.

In Japan, enzyme hydrolysis is used to produce a product called bio-fish meal (Uchida et al., 1990). This meal is produced from sardines by a low-temperature process (<75°C) and is used in fish feed and as a milk substitute for calves and piglets. A firm in Kashima outside Tokyo is producing this product, and production capacity is 150 tonnes per day.

Experiments with rats have found that fish protein hydrolysate made from good raw material has about the same nutritive value as casein (Higashi et al., 1965; Rebeca et al, 1991). Such products are, however, best suited as sources of protein for young animals with digestive systems that are not yet fully developed. This may apply to calves and piglets as well as fish larvae and fry (Mackie, 1982; Uchida et al., 1990). There are indications that the addition of a little fish protein hydrolysate to the feed for adult animals may be favourable

because it acts as an appetite stimulant or attractant, and because it may have immuno-stimulating effects.

Enzyme hydrolysis of stickwater is a process which has been developed to reduce drying costs in the fish meal industry. Since the removal of liquids by evaporation is cheaper than drying, it is important to obtain the highest possible content of solids in the stickwater concentrate. This process, which was developed by Novo Industrier A/S in Denmark, has been described in detail, both on a pilot scale and on an industrial scale (Jacobsen and Lykke-Rasmussen, 1984; Jacobsen, 1985; Christensen, 1989). At the second stage of the evaporation process, where the temperature is kept at 50-55°C, the bacterial enzyme preparation Alcalase is added to the stickwater. This enzyme treatment reduces the viscosity and burning-on in the evaporator so that the content of solids in the concentrate can be increased from 60% to about 70%. There is clearly a financial gain through energy saving. Even if Alcalase has produced the best results, cheap plant enzyme preparations can also be used with acceptable results (Schaffel et al, 1989).

The nutritional significance of this enzyme treatment is uncertain. Feeding experiments involving chickens indicate that

growth is somewhat less if concentrate of enzyme-treated stickwater is used in a feed mix instead of untreated concentrate (Hulan and Proudfoot, 1987).

15.

In agricultural villages along the coast, fish waste has always been used as fertilizer. While it seems to be a misuse of resources to use fish protein hydrolysate for such purposes, it should be mentioned that very interesting results have been obtained using such preparations in vegetable growing in the U.S.A. (Wyatt and McGourty, 1990). Apart from stimulating the growth of many plants, it has been found that the addition of fish protein hydrolysate may retard aging of peas and lettuce and delay the flowering and fruiting of tomatoes. The latter may be very appropriate in areas with frost problems in the spring. It has also been found that tomato plants which have been sprayed with fish protein emulsion can better tolerate being transplanted outside than can untreated plants.

Based on the results reported here, the conclusion can be drawn that fish protein hydrolysates have many uses and the raw material, hydrolytic enzymes and hydrolysis conditions must be selected based on the intended usage of the product. A long period of hydrolysis and high enzyme addition produce the

greatest yield, best water solubility and the highest content of important amino acids such as tryptophan, phenylalanine and cystein in the hydrolysate fraction. A high degree of hydrolysis will, however, produce a high content of bitter peptides and poor functional properties. A high degree of hydrolysis may also result in reduced nutritive value, particularly if the hydrolysate is used in high concentrations in fodder for adult animals having fully developed digestive systems.

In the manufacture of feed products and microbial peptones, the yield and nutritive value is of central importance and long periods of hydrolysis where both endogenous and exogenous enzymes are acting together will most often be advantageous. Alcalase is an enzyme preparation which produces a high yield by hydrolysis of fish proteins.

Plastein is a protein substance which can be produced by reversing the hydrolysis after the concentration of a protein hydrolysate (Montecalvo et al., 1984). Plastein has other functional properties and often has a higher content of essential amino acids than the protein in the original material (Onoue and Riddle, 1973). Briefly, the method is to produce an enzyme hydrolysate which is then concentrated to about 40% solids before

the plastein synthesis is initiated by the addition of new enzymes. Normally, the hydrolysate is produced by the use of pepsin at a low pH (about 2), while the plastein reaction is best at pH 4-5, using pepsin, papain, chymotrypsin or microbial proteinases as catalysts. In research, plastein has been produced from fish waste (Onoue and Riddle, 1973; Montecalvo et al., 1984; Raghunath and McCurdy, 1991), sardines (Ooshiro et al, 1981; Kim and Lee, 1987) and marine algae (Yamashita et al, 1976). It is, however, not known whether there is any industrial production of plastein from marine raw material. 16.

4. RIPENING BY THE ADDITION OF ENZYMES

Several hundred thousand metric tonnes of cured fish products are produced annually. The most significant of these are the herring products. Cured herring products have been produced for more than 1,000 years in the North Sea nations (Stefansson and Steimgrimsdottir, 1990). Traditionally, ripening is done by placing whole or headless fresh herring, mixed with salt, in barrels and storing cool for some months before filleting and packaging. During this storage, some of the fish's digestive enzymes are diffused out into the fish meat and there partially hydrolyze the muscle proteins, so that the muscle becomes soft and flexible. Enzymatic hydrolysis also causes freeing of peptides and amino acids which results in a pleasantly flavoured product. The high salt concentration (12-15%) inhibits the activity of the enzymes so that the ripening process is very slow and it will normally take about 2-7 months for ripening to be satisfactory (Anon, 1975; Opshaug, 1983).

The addition of enzymes in herring ripening has been experimented with since the early 70's in countries such as the Netherlands and Norway (Ritskes, 1971; Ruiter, 1972; Skåra, 1990).

There are several reasons why the addition of enzymes is desirable. It is primarily the trypsin-type enzymes from the pylorus sacs that are active in the ripening process. The quantity of these enzymes varies sharply with the time of year. As far as North Sea herring is concerned, only herring caught during the period May to August contains sufficient enzymes for a satisfactory ripening process (Ritskes, 1971; Anon, 1975). If enzymes are added, good ripening can also be achieved with herring caught at other times of the year. Ritskes (1971) has shown that good quality Matje-herring could also be produced from herring caught in early April if the herring had porcine or bovine pancreatic enzymes added. About 1 g of commercial enzyme preparations per kilo of herring was used. Enzyme preparations with a high lipase content produced an unwelcomed flavour. Ruiter (1972) achieved good and rapid ripening of spawned North Sea herring caught in October by adding ground pylorus from summer-caught herring.

18.

The long storage period in traditional ripening requires large storage capacity at controlled cooling. When putting down whole and headless herring, a large portion of the volume will also be taken up by the by-products. The fillets make up only half of a herring's round weight, and the by-products from the

filleting of ripened herring has such a high content of salt that they cannot be used for animal feed. There are therefore many reasons why direct ripening of fillets or gutted and headless herring is preferable to laying down whole fish. There are several patents which describe such processes.

A British patent (Anon, 1975) describes the production of Matje herring by adding 5% salt and 2.5% sugar to gutted and headless herring together with an enzyme preparation which contains mainly trypsin and chymotrypsin. Satisfactory ripening was achieved after 3 weeks' storage at 5°C, while the normal storage period for such products is 2-3 months. It was emphasized that the enzyme preparation must not contain pepsin and must have a low lipase content for the development of flavour to be satisfactory.

Opshaug (1983) describes a process where herring fillets are ripened by placing one part by weight of fillets in 1.3 parts by weight of brine containing 5% ground herring pylorus, 12% salt, 6% sugar and a mixture of spices. The fillets had a consistency and aroma corresponding to traditional pickled herring after only 5 days' storage at 3°C. Using this method, the salt concentration in the finished product will be only about

6% and it can therefore be consumed without first being soaked in water. The amount of pylorus used in this method corresponds to the amount normally found in herring which is to be ripened. The producer will therefore, by and large, not have to obtain ripening enzymes from other sources. A Canadian patent also describes the ripening of herring by adding fish viscera enzymes (Eriksson, 1975).

It is known that a German firm, Kordts KG, manufactures additives intended for rapid-ripening of herring fillets. One product, Komazyme, is a preparation of herring enzymes, while the other product, Komareif, is probably a starter culture of lactic acid bacteria mixed with carbohydrate substrate. In the sales brochure it is claimed that good Matje herring can be produced in only one week if these two products are added, together with a suitable amount of salt.

19.

At present, there are at least three Norwegian firms producing herring products by the rapid-ripening method (Kvåle, 1988). A/S Frøya Fiskeindustri uses an enzyme preparation manufactured in Germany. Frigopan A/S has used homogenized pylorus from fish as described by Opshaug (1983), but is now probably employing another method. Halvard Lerøy A/S employs a

method he developed himself. Together, these firms probably produce about 700 metric tonnes of rapid-ripened herring fillets per annum. The size of such production abroad is unknown.

In traditional slow-ripening methods, most of the enzyme activity will disappear by the end of the storage period. In rapid-ripening, the finished fillets will, however, still contain a fair amount of enzyme activity. This causes poor storage stability. The activity can be arrested by freezing the fillets, but the keeping quality after thawing is low because the enzymes continue to act (Skåra, 1990).

Simpson and Haard (1984) used pure bovine and Greenland cod trypsins in a ripening experiment with herring and squid. A clear ripening reaction was recorded where about 20 mg enzyme per kilo of raw material was added. At 10°C, the effect of the cod trypsin was stronger than the effect of bovine trypsin, but the cod trypsin lost its activity more rapidly than the bovine trypsin. Both these observations are in accordance with characterization of cod trypsins as low-temperature enzymes (Asgeirsson et al, 1989), and indicate that fish enzymes are better suited to rapid-ripening than enzymes from warm-blooded animals, both because

they act better at low temperature and because they can more rapidly be inactivated in the products during storage.

In the 1980's, Hermetikkindustriens Laboratorium (The (Norwegian) Canning Industry's Laboratory) studied rapid-ripening of sour pickled herring (Slinning, 1985 and 1986). No satisfactory results were obtained, the main problem being to achieve satisfactory storage stability of the end-product.

Norconserv is involved in trying to arrive at defined conditions which can provide a controlled rapid-ripening process and stable end-products (Skåra, 1990). Herring fillets are ripened by adding digestive enzymes from mackerel or cod together with different bacterial cultures. Promising results have been obtained, but the stability of the end-product is still not good enough. In this case, the addition of protease inhibitors from plants was considered. It is known that such inhibitors can easily be extracted from, among other things, the processing liquid from the potato industry. SSF has already carried out some promising experiments with stabilization of catches of bait-containing sand eel for fish meal production with the help of these inhibitors (Aksnes, 1991).

20.

The advantages of rapid-ripening are obvious. Filleting

prior to salting allows the by-products to be used directly as, for example, fish feed. Shorter production time reduces the need for refrigeration capacity. The use of less salt makes the soaking stage unnecessary. Active addition of enzymes also makes it possible to produce ripening products even from herring caught in periods when the fish naturally has a low content of digestive enzymes. If the problem of stabilizing the finished products could be solved satisfactorily, then such methods would undoubtedly have good future prospects.

And, finally, it should be added here that ripening by the addition of enzymes can also be used to develop new products - most likely from raw material which has not so far been tested for such purposes.

5. SELECTIVE HYDROLYSIS

Some enzymes can break down certain tissue structures in raw material without attacking other structures. Some tissue structures which are not normally broken down by a given enzyme can also be made open to degradation by this enzyme, provided chemical conditions such as temperature, degree of acidity and salt concentration are altered. These two factors make it possible to use enzymes as specific tools in the processing process where the purpose is selective degradation or alteration of certain tissue structures.

The oldest example of the use of such methods on marine raw material is probably the tanning of fish skin (Kjell Midling, (in conversation). Here, pancreatic enzymes are used to break down elastin and keratin structures in the skin while the main protein, collagen, is not broken down by the enzymes. The same method is also used in the tanning of skin from mammals. Today, tanning of fish skin is of little commercial importance, but there is an increasing interest in individual specialty products made from fish leather (Lewis, 1990).

Since the 1970's, and particularly in Norway and Iceland,

considerable research has been carried out to develop bio-technological methods for use in fish processing (Raa, 1986 and 1988; Stefansson, 1988; Stefansson and Steingrimsdottir, 1990). The reason is primarily that existing mechanical methods often damage the raw material. Sometimes the reason is also that there is no usable mechanical method which can do the job. Such an example is the removal of the black film from the sounds of cod. Salted sounds are produced in Iceland for export to countries in Southern Europe and the annual production is 30-50 metric tonnes. The market wants the product to be as white as possible and it is impossible to completely remove the black film using mechanical methods. Therefore, a method has been developed which makes it possible to remove the black film by a brief (20 min.) enzymatic hydrolysis (Stefansson and Steinmgrimsdottir, 1990). Icelandic researchers have also developed a method for enzymatic removal of the membrane which encloses the cod liver, but this method is not yet in use in the industry (Stefansson and Steingrimsdottir, 1990).

22.

A biochemical method of skinning herring has been developed in Norway (Norderhus et al., 1985 a). In this method, the herring is treated in a bath of 5% acetic acid at 10°C before it is transferred to an enzyme bath. The enzyme bath must have a

temperature of about 20°C and contain 0.5% acetic acid and 0.03% of a commercial fish pepsin product. The duration of the enzyme bath is 1-2 hours, depending on the size of the herring and its condition. Large herring must be treated longer than small herring, because the skin is thicker and the collagen structure more strongly cross-linked in the larger herring. Collagen is the dominant protein and the most important structural component in skin. This protein is resistant to most of the proteinases as long as it is in its natural form. Collagen is, however, easily denatured by acid or heat treatment and can then be easily broken down by most proteinases. A brief exposure to 5% acetic acid produces effective denaturing and the skin can be broken down by fish pepsin in low acidic conditions. Muscle protein is broken down relatively slowly under such conditions (Gildberg and Raa, 1979). This skinning method has been developed on a pilot scale but, so far, has not been used by industry. A similar method of scaling haddock is under development at Fiskeriforskning (Fisheries Research, Norway), and there is also interest in using such methods for skinning tuna, a product of great commercial importance (Raa, 1989).

One problem with any method that includes acid treatment is that it should preferably be carried out on round fish. If fish

muscle is exposed to acid, surface denaturing will occur which leads to contraction of the muscle fibres. This produces an appearance resembling that of a boiled product. In addition, when exposed for a long period of time, the acid will penetrate the muscle and produce a flavour change.

Icelandic researchers have developed an enzymatic method of removing skin from skate wings under neutral conditions (Stefansson, 1988; Stefansson and Steingrimsdottir, 1990). There exists mechanical equipment for such skinning, but the method is not efficient enough and skin remnants must be removed manually. With the enzymatic method, the collagen in the skin is heat-denatured so that it can be broken down by an enzyme mixture containing proteinases and carbohydrases. The enzyme treatment must continue for some hours at low temperature (below 10°C) before the skin remnants can be washed away. The process is not yet in use industrially (Stefansson and Steingrimsdottir, 1990). A US patent describes a similar method for removing the skin from tuna. (Fehmerling, 1973). 23.

The University of Tromsø and the firm Biotec Mackzymal have developed a method of skinning squid (Raa, 1986; Nilsen et al., 1989). The rubbery skin which covers the inner and outer surface

of the mantle and tentacles has a chemical structure which is fundamentally different from the muscle. It has therefore been possible to find an enzyme which selectively breaks down this skin, but which has little effect on the muscle (Raa, 1986). This method has been developed into an industrial process and is, briefly, as follows.: After gutting and washing, the mantle and tentacles are treated in an enzyme bath for two hours. The material is then washed and heat treated for 15-20 seconds at 80-90°C. Finally, the products are rinsed with cold water. The rubbery skin structure is now gone and the products can be vacuum-packed and frozen (Wray, 1988; Nilsen et al., 1989). Figure 1 is a picture of the industrial processing line.

24.

A US patent (Fehmerling, 1973) describes a method of enzymatic cleaning of mussels. Both Norway and Iceland are working on similar methods (Stefansson, 1988). However, it has proven to be difficult to create conditions which selectively free the muscle without it being softened by the enzymes (Stefansson and Steingrimsdottir, 1990).

In Norway, experiments have been carried out using enzyme treatment of shrimps to increase the yield and level of cleaning

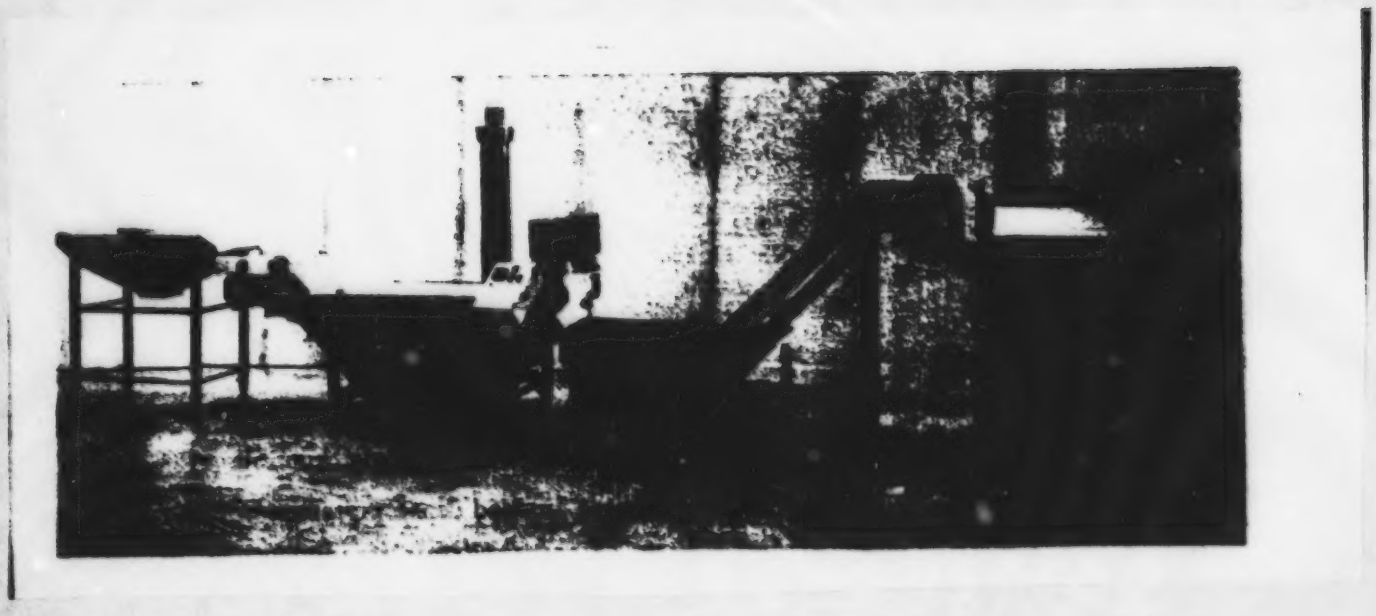

Figure 1. Processing line for enzymatic skinning of squid, developed by Biotec-Mackzymal in Tromsø and produced by Carnitech in Denmark.

by mechanical peeling (Norderhus et al., 1985b); Prytz and Stenberg, 1987). A number of proteolytic enzymes were tried, but no increased yield was recorded after enzyme treatment. Treating the shrimps with bromelain for 90 minutes at 25°C produced a better degree of clean-peeling than in the case of unripened shrimp, but it was found that ripening shrimp on ice for 20 hours produced a better result than any of the enzyme treatments.

Traditionally, mechanical methods are used for the production of caviar. Such methods are poorly suited to cleaning the roe from fat types of fish because much of the roe will be damaged or ruined. The caviar yield is often less than 50%. An American patent describes how proteinases can be used to free salmon roe from the connective tissue in the roe sac (Sugihara et al., 1973). It is claimed that proteinases, active in acid, neutral and basic environments, can be used. Norwegian experiments have found that common commercial proteinase preparations attack the roe's cell wall so that the quality is reduced. However, it has been possible to produce a fish enzyme preparation which selectively splits the bonds which fasten the egg cells to the connective tissue in the roe sac without attacking the cell wall of the roe (Raa, 1986). After he enzyme

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treatment, the roe can then be separated from the connective tissue by sedimentation and flotation in a tank. The connective tissue floats to the top of the tank while the roe settles on the bottom.By accurate control of the salt concentration, damaged roe can even be floated off. Using this process, it is possible to achieve more than 90% yield of undamaged roe (Raa, 1986; Wray, 1988). This method has been developed to industrial scale and, in 1990, a total of about 50 metric tonnes of salmon and trout caviar were produced in the Nordic countries and Australia. The prospects for this production to increase in the years to come appear good, particularly since the Soviet Union, among others, is also starting up production. Figure 2 shows a picture of the production equipment.

26.

Roe from some types of fish has a tendency to clump together after spawning. This results in a reduced oxygen supply and a reduced hatching yield. This clumping is caused by a mucoprotein layer which lies on the outside of the chorion membrane. If the eggs are treated with proteinases, then the mucoprotein layer can be peeled off so that clumping ceases. Experiments in the U.S.A. with roe from walleye have shown that treatment with bovine trypsin-type enzymes increased both the hatching yield and the degree of survival among the fish larvae after hatching (Krise et

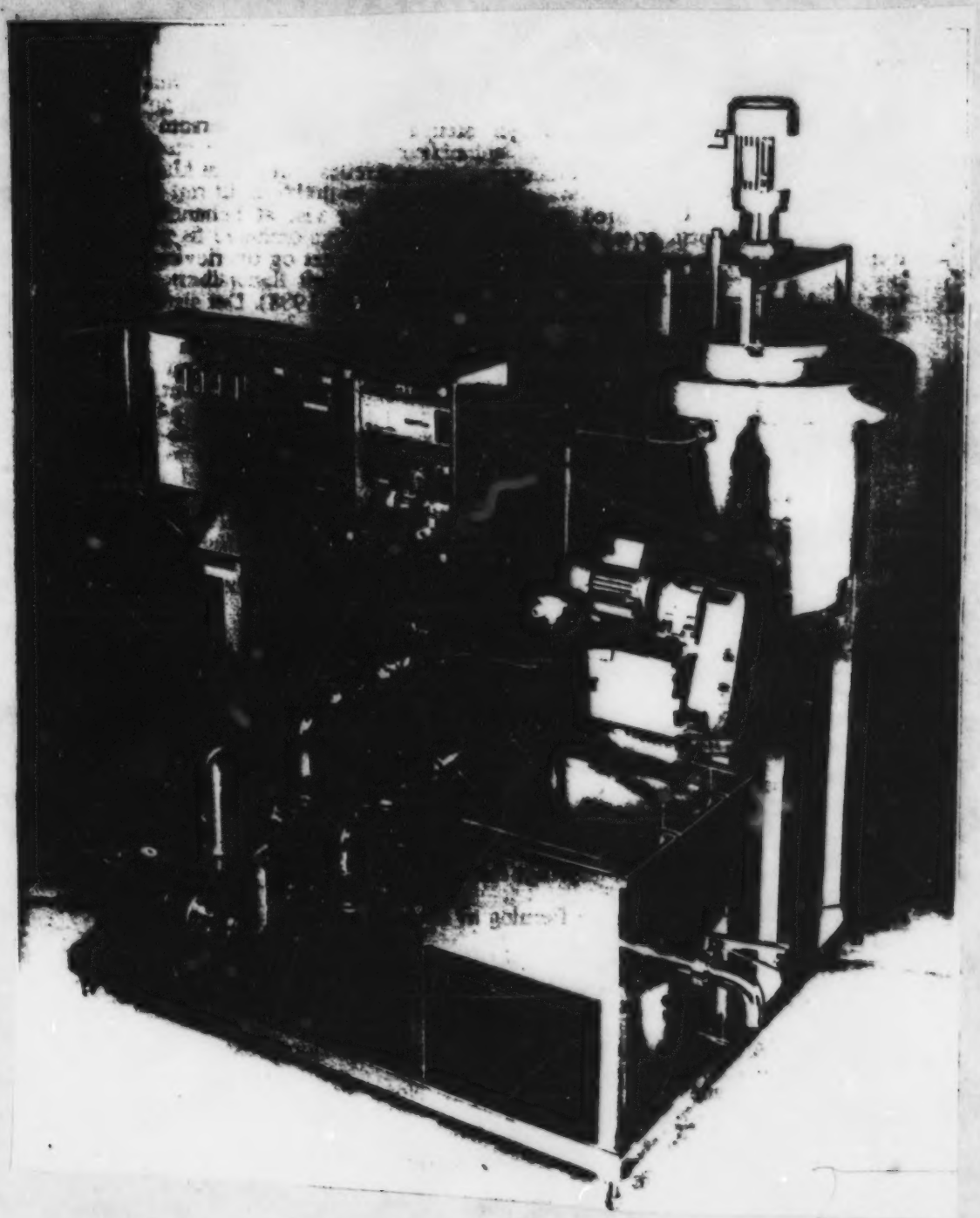

Figure 2. Processing equipment for enzymatic roe cleaning, developed by Biotec-Mackzymal in Tromsø and produced by Trio Industrier in Forus.

al., 1986; Krise, 1988). The latter is probably because enzyme-cleaned eggs are less vulnerable to fungal infections than eggs with the mucoprotein layer intact. The treatment consists of carefully stirring the eggs with a turkey feather in a bath which contains 0.01% enzyme preparation from bovine pancreas (degree of purity of the preparation is probably 50%) before washing with water. A similar method has also been developed for the treatment of lumpsucker roe (Horvath, 1980. Here Alcalase was used.

Enzyme treatment has also become important in the transfer of genetic material to fish eggs. Before genetic material can be injected into newly fertilized eggs, not only the mucoprotein layer, but also the chorion membrane itself must be removed. This is difficult to achieve without damaging the eggs. In controlled proteinase treatment, however, good results have been achieved (Hallermann et al., 1988). Different enzymes and conditions must be used for various types of fish eggs. Trypsin (2.5mg/ml) has been found to be quite suitable for gentle removal of the chorion membrane of goldfish eggs.

There is a market, particularly in France, for DNA extracted from cod or herring milt. DNA is normally extracted by chemical extraction processes which lead to the nucleic acids being

partially broken down. Some products will contain high-polymerized DNA and this can only be extracted with great care. Enzymes (papain preparations) are therefore used to break down protein before DNA is extracted (Erling Sandsdalen, in conversation).

Japanese researchers have found that a serin proteinase from an Aeromonas bacterium has the ability to selectively break down the byssus threads in blue mussels (Dohmoto and Miyachi, 1991). The practical significance of this discovery is unknown.

27.

Selective hydrolytic methods have still not reached great industrial importance in the processing of marine raw material. Of the enzymatic methods described here, only roe cleaning, squid skinning and cleaning of cod sounds have resulted in the development of industrial processes. The change from purely mechanical processes to biotechnological processes requires expertise which is not normally available in the traditional fish processing industry. Such methods also require great accuracy and good control of processing conditions. It is also often necessary to develop new processing equipment. All these factors make conversion to biotechnological methods a laborious and demanding process which takes a long time. However, it is quite evident

that the fish processing industry is nowadays increasing its expertise in this field. Therefore, there is reason to believe that conversion to such methods will be easier in the future, provided it can be documented that such conversion will produce product improvements or increased profitability.

6. VARIOUS METHODS

In this chapter I will briefly describe some of the methods which fall outside the areas so far described, as well as methods which can be said to be somewhat peripheral to the theme of this report.

By-products from shellfish production contain much of the polysaccharide chitin. It is estimated that this amount of chitin constitutes 120,000 metric tonnes on an annual basis (Knorr, 1991). Chitin and chitin derivatives are used in quite a few areas, but enzymes are used to only a small extent in the extraction and processing of chitin. In the U.S.A., however, a method has been developed for the enzymatic degradation of chitin to its monomer, N-acetylglucosamine (Carroad and Tom, 1978; Revah-Moiseev and Carroad, 1981; Cosio et al., 1982). Once the offal has been cleaned of proteins and minerals by chemical methods, some of the chitin is used as growth substrate for a mutant of the bacterium Serratia marcescens which produces a large amount of chitinase. The chitinase is secreted and the culture filtrate is used as an enzyme preparation to hydrolyze the majority of the chitin. When chitin from shrimp shell was used, the hydrolytic yield was 80% after a 24 hour's hydrolysis at 30° C

(Cosio et al, 1982). The hydrolytic product, N-acetylglucosamine, was then used as a growth substrate in the cultivation of yeast for the production of unicellular protein for animal feed. Growth experiments were carried out using more than 40 types of yeast and Pichia kudriavzevii was found to be best suited because it grew well on an single growth medium at low pH (4-5) and at a relatively high temperature (37°C). Yeast is well suited as an additive in fish feed. In addition to being a good source of protein and vitamins, yeast cell components can also stimulate the nonspecific immuno-defence system and thereby increase the fish's general resistance to diseases (Raa et al, 1990). From a purely market economy point of view, this process is not profitable in the U.S.A. (Cosio et al, 1982), but it should also be evaluated as a contribution to reducing a large pollution problem. Chinese researchers have also worked with the enzymatic degradation of chitin from marine shellfish (Xiuli et al, 1989).

29.

Chitosan is produced by chemical deacetylation of chitin. Chitosan is a biopolymer which is used for both medical and biotechnological purposes, such as purifying water and clarifying beer. An important future prospective is that this polymer is degradable by micro-organisms and can therefore be used in the

production of environmentally friendly packaging (Knorr, 1991). Under chemical deacetylation, chitin, which has a molecular weight of 160,000, is split into chain lengths shorter than 50,000. This may be inappropriate since a high molecular weight is essential for many applications. There are, however, enzymes which can deacetylize chitin without reducing the chain length (Nestaas, 1986). It is not known whether such enzymes are used in the industrial production of chitosan.

The carotenoid astaxanthin is the most important pigment in shrimp. American researchers have found that this pigment can easily be extracted from boiled shrimp waste if trypsin is added to the extraction buffer (Cano-Lopez et al., 1987). The best yield (64%) was obtained when cod trypsin was used at 4° C. Astaxanthin is also the natural pigment in salmonids and therefore very valuable as an additive in feed for such fish. A method has also been developed for the extraction of astaxanthin from crayfish (Chen and Meyers, 1983). Here, the effect of the raw material's own enzymes at pH 4.5-5 is utilized before the pigment is extracted with hot oil (85-90°C).

The health aspects of polyunsaturated fatty acids from marine oils are well known. There is great interest in producing

products enriched with these acids. This can be achieved by, among other thing, the use of lipases. Haraldsson (1990) has presented a summary of the use of lipases in the modification of marine oils. The main principle of such enzymatic methods is that some polyunsaturated fatty acids are poor lipase substrates. This applies particularly to the important acid DHA (docosahexaenoic acid).

Hoshina et al, (1990) found that the total content of DHA and EPA (eicosapentanoic acid), could be doubled in triglyceride fractions in oils from cod liver or sardines if the oils were treated with lipases from Candidae cylindracea or Aspergillus niger. Hills et al., (1990) found that DHA could be concentrated as much as five times in the fatty acid fraction by selective esterification of free fatty acids from cod liver oil. In the esterification, a commercial lipase, Lipozym (Novo Industrier A/S), extracted from Mucor miehei, was used. Danish researchers used the same enzyme to concentrate DHA from sand eel oil (Langholz et al, 1989). Free fatty acids or fatty acid methylesters were used as enzyme substrates. In both cases, DHA enriched that fraction which was not modified by the enzyme. Lipozym was also used by Icelandic researchers who found that the

30.

EPA and DHA content in cod liver oil could be increased from less than 20% to around 50% by enzymatic inter-esterification (Haraldsson et al., 1989).

Even though EPA and DHA are not normally good lipase substrates, lipases have been found which quite effectively hydrolyze triglycerides with polyunsaturated fatty acids. Cromobacterium viscosum is a bacterium which synthesizes such a lipase. This lipase is produced commercially and is called Lipase TOYO (Osada et al, 1990). It has been found that EPA and DHA are freed quite effectively from triglycerides in sardine oil by using this lipase. Similarly, the lipase can be used to catalyze the reversed reaction to produce triglycerides with a high content of EPA and DHA.

Some raw materials have naturally quite high contents of polyunsaturated fatty acids. About 10% of the solids in cod roe is phospholipids and about half of these consist of polyunsaturated fatty acids. In hydrolysis of fat from cod roe by lipase from porcine pancreas, a fatty acid hydrolysate was obtained which contained 24% EPA and 40% DHA (Tocher et al, 1986). Laboratory experiments showed that 60% of the

polyunsaturated fatty acids in cod roe could be extracted by this method, that is, a yield of about 6 g polyunsaturated fatty acids per kg. of cod roe.

Polyunsaturated fatty acids are very vulnerable to oxidation. It is known that enzymes with antioxidative effects can be used to stabilize marine oil (Anders Aksnes, in conversation).

There is today a considerable production of marine oils enriched with polyunsaturated fatty acids. It is uncertain if enzymes are used to any great extent in such production. The health aspect of using such concentrates instead of natural oils is widely discussed. It is therefore difficult to predict what the importance of such products will be in the future. 31.

Marine algae, such as seaweeds, are raw material for the production of a number of interesting polysaccharides used in the foodstuffs industry, fish feed and cosmetics, as well as in biotechnological methods for separation and cell culture (Weiner et al., 1985). It is not known whether enzymes are being used in industrial processing of marine algae (Bjørn Larsen, in conversation). However, enzymes are being used in research to produce, among other things, protoplasts for the cultivation of

tissue cultures. Here, mainly enzyme preparations from bacteria and snail (abalone), possibly in combination with pectinase, sulfatase or macerase, are used (Polne-Fuller et al., 1984; Knutsen, 1991). The gene for an elastase-like enzyme from the abalone has been cloned, and future use of this enzyme in the processing of marine algae has been indicated (Gropp and Morse, 1989).

7. CONCLUSION

The active use of enzymes in the processing of marine raw material has so far been limited. The greatest importance of the enzymes is still in traditional ripening and autolytic processes where the enzymes are a natural part of the raw material. The most important areas in which such methods are used are the production of fish sauce and silage from small fish, by-catch fish or fish waste and in the ripening of herring products.

The active use of enzymes is most important in the production of fish protein hydrolysates. Such products can be roughly divided into two categories. Consumer products which must have good flavour and, preferably, quite good functional properties, and feed products or growth substrates for micro-organisms, where high hydrolytic yield and good nutritive values are the most important criteria. The consumer products are usually produced by controlled brief hydrolysis under gentle conditions. The plant enzyme bromelain is often preferred in such processes. In the production of feed products, extensive hydrolysis with microbial enzyme preparations, such as Alcalase, will often be appropriate.

Selective enzyme hydrolysis as an alternative to mechanical

separation is not widely used at present. It is not easy to get the traditional fish processing industry to convert to such processes without a clear financial gain being documented. Such documentation requires expensive trials on a full industrial scale. Since the fish processing industry is not very capable or willing to contribute to meeting such expenses, the development normally stops at the pilot study stage. Two or three enzyme processes in this category have, however, been taken into use commercially, and these will become important touchstones for the utilization of biotechnology as an alternative to purely mechanical technology in the fish processing industry. The results have been very positive so far, but it has been found that the requirement for technical follow-up and control is high. Increased technical expertise in the industry is a prerequisite to such methods becoming generally widespread in the future.

33.

Relatively small financial resources have been used to develop the enzyme technology employed on marine raw material. This is the main reason why Norway is among the leading nations in this field. Norwegian research and development has left its mark on four fields in particular:

1) ensilage technology

2) rapid-ripening of herring

3) selective hydrolysis of tissue structures

4) production of fish peptone

The ensilage technology is now fully developed on an industrial scale and is, by and large, managed by competent people in the industry. However, the other three fields are still in the developmental stage and therefore need considerable support from the research and consulting environments.

Rapid-ripening of herring is a principle which is a clear improvement over traditional methods, provided process control and stabilization of the end-product can be improved. The most important task here will be to find an effective method of stopping enzymatic effect without decreasing the quality of the product. The chance of success appears to be greatest if fish enzyme preparations are used. The development of simple and inexpensive methods for the production of defined fish enzyme preparations will therefore be an important part of this work.

The production of fish peptone is an interesting field with many future prospects, not only for use in the cultivation of micro-organisms, but also for use as a source of protein and as an attractant in starter feed for fish. Peptone products made

from fish viscera or whole fish appear to have special health properties. There are several indications that such products have nutritional and medicinal properties which cannot be explained based on simple chemical analyses (Christoffersen, 1977). The great challenge here is to discover more about this and to identify which factors may be essential. The next step could therefore be to optimize the hydrolysis process with regard to such factors.

34.

Selective tissue hydrolysis is a field where new problems will continually appear as new raw material and new types of products become of interest. Norway and Iceland are clearly ahead in research in this field, and it is important that this research be intensified in the future if this position is to be retained. Heavy investments in marine biotechnology are being made in countries such as Japan, the U.S.A. and Canada and there is a very great danger that we will easily be left behind. It is therefore very important for us to concentrate our resources on those fields where we already hold a leading position.

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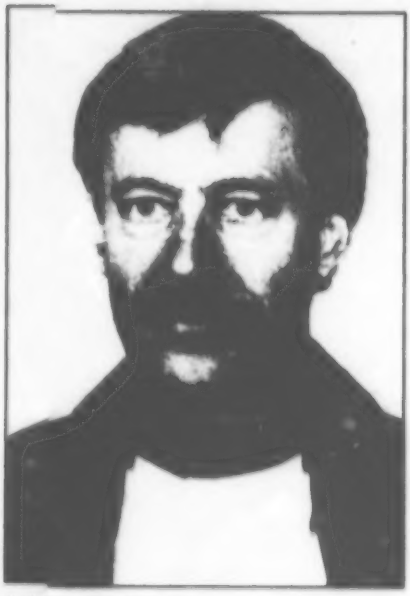

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